DNA amplification with partial-contact heating

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The Spartan DX amplifies DNA products with the same accuracy and reliability as conventional fixed-temperature and ramping-temperature thermal cyclers, and with equivalent or faster run times.

Introduction

Real-time PCR has substantially increased the efficiency of DNA testing by reducing reaction times, increasing sensitivity, and eliminating the need for cumbersome electrophoresis equipment for amplicon detection (Ref 1). The Spartan DX is a real-time PCR device designed for on-demand DNA applications. It makes use of fixed-temperature heat blocks that transfer heat by partial contact to the reaction tubes. Heating by fixed blocks enables much faster temperature transitions than Peltier-based heating and cooling (Ref 2). For example, the RoboCycler® 96 from Stratagene uses fixed-temperature heat blocks and can achieve run times that are twice as fast as the GeneAmp® PCR System 9700 from Applied Biosystems, a thermal cycler that ramps the temperature of its heat block using Peltier-based technology.

The purpose of this study was to determine if the Spartan DX, using partial-contact heating, performs accurate and reliable DNA amplification when compared to conventional fixed-temperature and ramping-temperature block thermal cyclers.

Materials and Methods

DNA extraction

DNA was isolated from 10 clinical isolates of *Staphylococcus aureus.* For each isolate, 4-5 medium-sized bacterial colonies were resuspended in 100 µl of Lysis Buffer (50 mM Tris-HCl, 50 mM NaCl, 5 mM EDTA, pH 8) with 2 µl of 1 mg/ml lysostaphin (Sigma-Aldrich, Cat No. l7386). The samples were incubated at 37°C for 30 min. Following this incubation, 5 µl of 20 mg/ml proteinase K (Sigma-Aldrich, Cat. No. p2308) was added to the mixture and the tubes were shaken at 50°C for 1 h. The tubes were then incubated at 100°C for 10 min to inactivate the proteinase K. Extracts were stored at 4°C.

PCR amplification

Oligonucleotide primers were designed against a conserved region of the bacterial *16S rRNA* gene. The forward primer sequence was 5'-cga aag cgt ggg gat caa ac-3', and the reverse primer was 5'-ccc agg cgg agt gct taa tg -3'. The primers were designed with Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The expected amplicon size was 125 bp. Components of the PCR amplification mixture are listed in Table 1.

Amplifications were performed on the Spartan DX, the RoboCycler 96 (Stratagene), and the GeneAmp PCR System 9700 (Applied Biosystems). For all runs, 0.2 ml thin-wall flat cap PCR tubes were used (Axygen, Cat. No. PCR 02C, PCR 02D), and reactions for the Spartan DX, or the RoboCycler 96 were topped with 15 µl of mineral oil (Biotools, Cat. No. 20.032) to prevent evaporation. Cycling parameters are listed in Table 2. For all three machines, a two-temperature cycling program was performed by combining the annealing and extension steps.

DNA analysis

Fluorescence values were downloaded from the Spartan DX to a computer and graphed using Microsoft Excel®. In addi-

Component	Final Amount
10X PCR Reaction Buffer (No $MgCl_2$) (Invitrogen)	1 X
$MgCl_2$ (Invitrogen)	2.5 mM
dNTP mix (Invitrogen)	0.125 mM
Taq DNA polymerase (Invitrogen)	1 U
SYBR Green I (Invitrogen)	0.5 X
PCR primers (Sigma-Aldrich)	1 µM each
Template DNA	2 µl
Sterile water	
Total reaction volume	**20 µl**

Table 1. Components in amplification mixture.

Step	Temperature	Time	Cycles
Initial denaturation	93.6°C	30 s	1
Denaturation	93.6°C	20 s	35
Annealing/extension	57.0°C	20 s	35

Table 2. Cycling parameters.

tion, all real-time PCR results were confirmed by agarose gel electrophoresis using 10 µl of the amplification products.

Results

Real-time fluorescence results from the Spartan DX were positive for all 10 samples and threshold cycle (Ct) values ranged from 9 to 19. A representative result is shown in Figure 1. Gel electrophoresis indicated that amplifications from all three machines generated specific amplicons of the correct size (125 bp) (Fig 2). Run times for the Spartan DX, RoboCycler, and GeneAmp 9700 were 28, 21, and 60 min, respectively.

Discussion and Conclusions

These results demonstrate that the Spartan DX is accurate and reliable for DNA amplification. Its performance is equivalent to conventional fixed-temperature and ramping-temperature thermal cyclers. The Spartan DX's real-time fluorescence detection saves time and labor compared to standard gel electrophoresis detection methods.

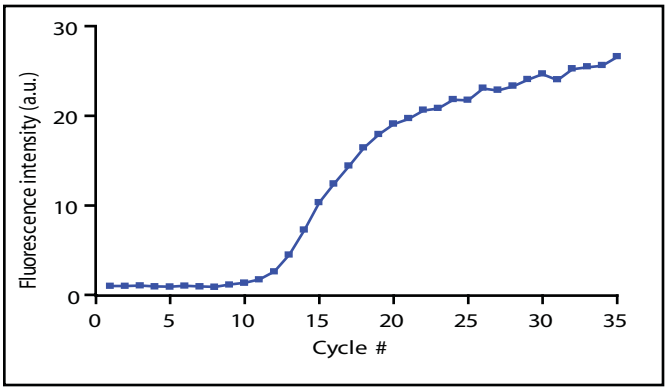

Figure 1. Real-time PCR result from the Spartan DX.

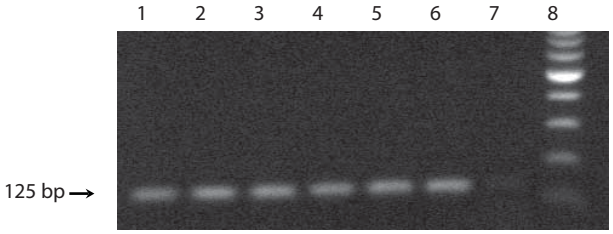

Figure 2. Gel electrophoresis analysis of PCR products. Lane 8 contains 100 bp DNA ladder (Invitrogen). Lanes 1,2 are samples run on the Spartan DX, lanes 3,4 are samples run on the Robocycler and lanes 5,6 are samples run on the GeneAmp 9700. Lane 7 is no DNA negative control. The expected band size of 125 bp is seen for all samples.

References

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